



HOSTED BY



ELSEVIER

Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

## Asian Pacific Journal of Tropical Medicine

journal homepage: <http://ees.elsevier.com/apjtm>Original research <http://dx.doi.org/10.1016/j.apjtm.2016.01.027>Regulating effect of activated NF- $\kappa$ B on edema induced by traumatic brain injury of rats

Zi-Ran Wang, Yu-Xin Li, Hong-Yan Lei, Dai-Qun Yang, Li-Quan Wang, Ming-Yu Luo\*

Department of Cerebral Surgery, Linyi People's Hospital, Shandong 276000, China

## ARTICLE INFO

## Article history:

Received 15 Dec 2015

Received in revised form 20 Dec 2015

Accepted 30 Dec 2015

Available online 15 Jan 2016

## Keywords:

Nuclear transcription factor- $\kappa$ B

Traumatic brain injury

Astrocytes

Rat

## ABSTRACT

**Objective:** To observe the effect of nuclear transcription factor- $\kappa$ B (NF- $\kappa$ B) on cerebral edema in rats with traumatic brain injury (TBI).**Methods:** Male SD rats with fluid percussion injury (FPI) were selected. After separation and culture, rats' astrocytes all suffered FPI. The expression of NF- $\kappa$ B and the water content were detected at the animal and cellular levels, while the activity of NOX was evaluated at the cellular level.**Results:** According to the results, the positive expression of NF- $\kappa$ B and expression of mRNA were significantly increased and the water content was increased for rats after TBI, while NF- $\kappa$ B inhibitor BAY11-7082 could significantly reduce the effect of TBI. 1 and 3 h after FPI of astrocytes, the activation of NF- $\kappa$ B was increased and BAY 11-7082 could significantly improve the injury-induced swelling of astrocytes. After the injury of astrocytes, the activity of NOX was also increased, while BAY 11-7082 could reduce the activity of NOX.**Conclusions:** The results show that the activation of NF- $\kappa$ B in astrocytes is a key factor in the process of cerebral edema after TBI of rats.

## 1. Introduction

Cerebral edema is major complication of nervous system for traumatic brain injury (TBI), and the swelling of astrocytes is the important part of early cerebral edema after the trauma [1,2]. The mechanism of astrocytes swelling after trauma has not been clear yet. It is considered that oxidative stress plays a key role in the pathogenesis of TBI [3–5]. According to the previous researches, the injury of astrocytes cultured *in vitro* could significantly increase the free radical [6].

The active oxygen free radical could stimulate the activation of different kinds of transcription factors, especially nuclear transcription factor- $\kappa$ B (NF- $\kappa$ B) [7,8]. The activated NF- $\kappa$ B might contribute to the swelling of astrocytes after being exposed to ammonia [9], and it also plays a role during cerebral edema after hepatic failure [10]. Meanwhile, the

activated NF- $\kappa$ B is found after TBI [11]. However, the function of NF- $\kappa$ B in the swelling of astrocytes and cerebral edema after TBI has not been explained. This study is to discuss the activation of NF- $\kappa$ B after the injury of astrocytes *in vitro* and *in vivo* and whether the activated NF- $\kappa$ B would contribute to the swelling of astrocytes and cerebral edema after TBI.

## 2. Materials and methods

## 2.1. Animals

Male SD rats with the weight of 250–350 g were provided by the laboratory of Shandong University. All rats were fed under sterile environment with the temperature of  $(22 \pm 2)^\circ\text{C}$ . Rats were given free diet and drinking.

## 2.2. Instruments and reagents

The fluid percussion injury device was purchased from NatureGene Corp.; the rabbit polyclonal NF- $\kappa$ B and GFAP antibodies from Santa; DMEM and fetal bovine serum from Gibco; 3-O-methyl-D-glucopyranose from Y-Y Chemical Reagents; NOX activity assay kit from Shanghai Suolaibao Bio-technology Co., Ltd.; BAY 11-7082 and SN50 from Sigma.

\*Corresponding author. Ming-Yu Luo, Master's Degree, Deputy Attending Physician, Department of Cerebral Surgery, Linyi People's Hospital, Shandong 276000, China.

Tel: +86 13954993801

E-mail: [nyjzlh@163.com](mailto:nyjzlh@163.com)

Peer review under responsibility of Hainan Medical College.

Foundation Project: It was supported by Technology Program for Development of Pharmacy of Shandong 2013 (No. 2013WS0351).

## 2.3. Methods

### 2.3.1. Establishment of rat model of moderate brain injury with lateral fluid percussion

The method proposed by Zhang *et al.* [9] was used to establish the rat model of moderate brain injury with lateral fluid percussion (TBI). The part of percussion was the right parietal cortex. Rats in Sham group were given the same procedure except the injury. Rats in the treatment group were given the intravenous injection of BAY 11-7082 (1, 5 and 10 mg/kg) and NF- $\kappa$ B inhibitor 5–10 min after TBI. Rats in other groups were given the equal volume of normal saline. Afterwards, rats were randomly divided into the control group, sham group, TBI group and BAY 11-7082+TBI group.

### 2.3.2. Detection of cerebral water content

Three hours after TBI, the head of rats was cut off and the brain was collected. The filter paper was used to absorb the water on the surface of brain at first. Then the brain was placed on the electronic analytical balance to analyze the wet weight. Afterwards, it was placed in the oven for the baking at 110 °C for 24 h and then the dry weight was measured. The cerebral water content (%) = (wet weight – dry weight)/wet weight  $\times$  100%.

### 2.3.3. Immunohistochemistry

The brain tissues were fixed with 4% paraformaldehyde for 24 h and then stored in the cold 30% sucrose for 12 h. Afterwards, they were cut into 20  $\mu$ m slices. After being sealed with the fetal bovine serum for 30 min, they were given the DAPI staining and then cultured with rabbit polyclonal NF- $\kappa$ B and GFAP antibodies for 3 h at the room temperature. After being washed with PBS for 3 times, they were incubated in the fluorescein-labeled horseradish peroxidase secondary antibody for 30 min. Then the slides were prepared and observed under the microscope.

### 2.3.4. RT-PCR to detect the expression of NF- $\kappa$ B in brain tissue

Trizol kit (Invitrogen) was employed to extract the total RNA from the brain tissue. RNA was reversely transcribed into cDNA through one-step RT-PCR kit and then PCR amplification was performed. The collected 5  $\mu$ L amplification products were used for the testing in 2% agarose gel.

### 2.3.5. Culture of primary astrocytes

After collection of the head from 1-day rat, the cortex was separated and sliced into pieces in HBSS. It was then digested with the pancreatin. The digestion was ended after adding 10% FBS. The supernatant was removed after centrifugation and then seeded on DMEM with the double-antibody and fetal bovine serum. Then it was cultured in the incubator at 37 °C and 5% CO<sub>2</sub>.

### 2.3.6. Establishment of fluid percussion injury (FPI) of astrocytes

The fluid was replaced according to the routine procedure 24 h before the fluid percussion. After eliminating the metabolic product of cells, the culture medium was removed. The alcohol was used for the disinfection around the Petri dish. It was then placed in the injury chamber that was full of Hanks. The falling

angle of hammer was adjusted to set the impact force as 0.2 MPa. After the percussion, the culture medium was added and it was placed in the incubator. NF- $\kappa$ B inhibitor BAY 11-7082 or SN50 was added in astrocytes after FPI. After being cultured for 3 h, the related detection was performed. Cells were divided into control group, sham group, FPI group, BAY 11-7082+FPI group and SN50+FPI group.

### 2.3.7. Detection of cell volume

One milliliter 3-*O*-methyl-D-glucopyranose was added in the culture medium that contained the injured cells. After the culture, the culture medium was collected and stored separately for the radioactivity measurement. Cells were washed rapidly. A total of 0.5 mL 1N NaOH was added to determine the radioactivity of cell extracts.

### 2.3.8. Western blot assay

To specify the changes in the expression of NF- $\kappa$ B after TBI, the moderate fluid percussion injury was applied to the right parietal cortex of rats. The cerebral cortex was cut into sections for the examination 3 h after TBI. To explore the changes of NF- $\kappa$ B in the astrocytes, the sections were labeled with GFAP and DAPI at the same time. The whole-cell lysis buffer was added in the cultured astrocytes according to the proportion of 10 mL/g. After the homogenization, cells were broken with the ultrasound. After all cells that had been lysed, BCA method was employed for the protein quantification. The gel electrophoresis and membrane transfer were performed. The equal samples were collected for 10% gel electrophoresis. Afterwards, PVDF membrane was taken out and sealed with 10% skim milk. The membrane was put in NF- $\kappa$ B antibody with the concentration of 1:1000 and then incubated at the room temperature for 3 h. Afterwards, the membrane was put in 1:5000 horseradish peroxidase labeled goat anti-rabbit or goat anti-mouse IgG to be incubated for 1 h. According to the reaction steps of ECL kit, it was exposed in the darkroom to obtain the X-ray film that indicated the specific protein bands.

### 2.3.9. Detection of NOX activity

After the lysis of cells, the activity of NOX in the supernatant was detected according to the steps of NOX activity assay kit.

## 2.4. Statistical analysis

The data was processed with SPSS 18.0. All data was expressed by the mean  $\pm$  standard deviation. The tukey *post-hoc* comparison was performed among groups.  $P < 0.05$  indicated the statistically significant difference.

## 3. Results

### 3.1. Expression of NF- $\kappa$ B in cerebral cortex of rats after TBI

According to the results of immunohistochemistry in Table 1, compared with Control group and Sham group, the positive expression of NF- $\kappa$ B was increased in TBI group, while 1, 5, and 10 mg/kg BAY 11-7082 could inhibit the positive expression of NF- $\kappa$ B in the cortex of TBI. According to the results of RT-PCR in Table 1, compared with Control group and Sham group, the expression of NF- $\kappa$ B mRNA was increased in TBI group,

**Table 1**

Expression of NF-κB and mRNA in cerebral cortex of rats after TBI.

Group	Dose (mg/kg)	Positive expression rate of NF-κB (%)	Expression of NF-κB mRNA
Control group		8.45 ± 0.78	0.21 ± 0.02
Sham group		8.46 ± 0.92	0.22 ± 0.03
TBI group		72.65 ± 7.31**	1.15 ± 0.10**
BAY11-7082 group	1	58.08 ± 6.11##	0.85 ± 0.09##
	5	43.45 ± 5.27##	0.64 ± 0.07##
	10	26.39 ± 2.70##	0.41 ± 0.04##

Compared with Control and Sham groups, \*\* $P < 0.01$ ; compared with TBI group, ## $P < 0.01$ .

while 1, 5, and 10 mg/kg BAY11-7082 could reduce the expression of NF-κB in the cortex of TBI.

### 3.2. Inhibition of NF-κB to reduce cerebral edema of rats after TBI

As shown in Table 2, compared with the control group and sham group, the degree of cerebral edema in TBI group was significantly increased, while the treatment of 1, 5 and 10 mg/kg BAY 11-7082 could reduce the cerebral edema after TBI, while the treatment of BAY 11-7082 (5 mg/kg) had the most significant effect.

### 3.3. Activation of NF-κB after FPI of astrocytes

According to the results of western blot shown in Table 3, compared with the control group, the expression of NF-κB protein was increased in FBI group and decreased in BAY11-7082+FPI group and SN50+FPI group. According to the results of RT-PCR shown in Table 1, compared with the control group, the expression of NF-κB mRNA was increased in TBI group and decreased in BAY11-7082+FPI group and SN50+FPI group.

### 3.4. Inhibition of NF-κB to reduce the swelling of astrocytes after FPI

As shown in Table 4, compared with the control group, the cell swelling was significant in FPI group, while the degree of cell swelling was decreased in BAY11-7082+FPI group and SN50+FPI group.

### 3.5. Inhibition of NF-κB to reduce the NOX activity of FPI-induced cells

As shown in Table 4, compared with the control group, the NOX activity was significantly increased in FPI group and decreased in BAY 11-7082+FPI group and SN50+FPI group.

**Table 2**

Effect of BAY 1107082 on cerebral edema of rats after TBI.

	Control group	Sham group	TBI group	BAY11-7082 group (mg/kg)		
				1	5	10
Cerebral edema (%)	20.28 ± 2.30	21.61 ± 3.79	80.4 ± 8.31**	62.40 ± 6.50##	32.10 ± 3.33##	42.10 ± 3.33##

Compared with Control and Sham groups, \*\* $P < 0.01$ ; compared with TBI group, ## $P < 0.01$ .

**Table 3**

Expression of NF-κB after FPI of astrocytes.

Group	Expression of NF-κB protein	Expression of NF-κB mRNA
Control group	0.48 ± 0.05	0.33 ± 0.03
FBI group	1.32 ± 0.14**	1.24 ± 0.13**
BAY11-7082+FPI group	0.67 ± 0.07##	0.75 ± 0.08##
SN50+FPI group	0.68 ± 0.08##	0.80 ± 0.09##

Compared with Control and Sham groups, \*\* $P < 0.01$ ; compared with TBI group, ## $P < 0.01$ .

**Table 4**

Inhibition of NF-κB to reduce the swelling and NOX activity of astrocytes after FPI.

Group	Cell volume (%)	NOX activity
Control group	100.00 ± 9.78	1.38 ± 0.14
FBI group	160.38 ± 16.10**	5.29 ± 0.52**
BAY11-7082+FPI group	122.64 ± 10.45##	2.64 ± 0.28##
SN50+FPI group	118.21 ± 11.37##	2.63 ± 0.25##

Compared with Control and Sham groups, \*\* $P < 0.01$ ; compared with TBI group, ## $P < 0.01$ .

## 4. Discussion

According to the results of this study, the positive expression of NF-κB, the expression of mRNA and cerebral water content were increased in rats after TBI, while BAY11-7082 could significantly reduce the cerebral water content after TBI. The FPI of astrocytes could also induce the activation of NF-κB and the inhibition against the activation of NF-κB could reduce the swelling degree of astrocytes caused by the injury. Furthermore, the inhibition of NF-κB could also reduce NOX activity of astrocytes that is caused by FPI. The results indicate that the activation of NF-κB in astrocytes is a key factor in the formation of cerebral edema after TBI.

The oxidative stress and inflammatory mediator could also activate NF-κB [12–14]. The activated NF-κB could induce the inflammation/oxidative stress response genes, which would play the key role in some neurological injuries [15]. The activated NF-κB is also found in the brain after TBI [16]. But it has not been clear that whether NF-κB is involved in the astrocyte swelling/cerebral edema after TBI of brain. The results of this study also indicates that FPI of astrocytes could cause the activation of NF-κB, while BAY11-7082, a kind of NF-κB inhibitor, could significantly reduce the FPI-induced swelling of astrocytes. The mechanism for the mechanical injury of astrocytes to activate NF-κB and the further cell swelling has not been clear yet. According to a study, the oxygen free radicals are increased after the injury of astrocytes and the mitochondria permeability transition pore (mPT) and

MAPKs are activated. The inhibition of mPT and MAPKs could relieve the swelling of astrocytes [6]. It is because the increased oxygen free radicals could activate NF- $\kappa$ B and induce the activation of mPT and MAPKs [17]. There are some other factors that contributed to the activation of NF- $\kappa$ B of course.

The mechanism of activated NF- $\kappa$ B in the astrocytes that are induced by the mechanical injury has not been clear. The oxidative stress is the potential factor for the activation of NF- $\kappa$ B. The activation of NF- $\kappa$ B could stimulate the generation of NOX, while NOX is one of major sources for the superoxides [18,19]. It has been proved that the activated NOX could contribute to the swelling of astrocytes after the treatment of ammonia [9], which is in accordance with the findings of this study. It's found that the activity of NOX is increased after the cultured astrocytes suffer from the mechanical injury, while BAY11-7082 could significantly reduce the activity of NOX. All above results show that the cell swelling after NF- $\kappa$ B-induced FPI is mediated by increasing the activity of NOX to the certain extent.

In conclusion, the activation of NF- $\kappa$ B and cerebral edema are significantly increased after TBI and the inhibition of NF- $\kappa$ B could significantly reduce the cerebral edema after TBI. The FPI of astrocytes could induce the activation of NF- $\kappa$ B and increase the activity of NOX. BAY11-7082 could significantly reduce the activity of NOX and the swelling of astrocytes. The results indicate that the activation of NF- $\kappa$ B in astrocytes play a key role in the formation of cytotoxic cerebral edema after TBI. The inhibition against the activation of NF- $\kappa$ B might be applied in the treatment of early cerebral edema for patients with TBI.

### Conflict of interest statement

We declare that we have no conflict of interest.

### References

- [1] Wang CG. Research advances in the pathogenesis of traumatic cerebral edema. *Chin J Brain Dis Reh* 2012; **2**(4): 30-32.
- [2] Kou Z, VandeVord PJ. Traumatic white matter injury and glial activation: from basic science to clinics. *Glia* 2014; **62**(11): 1831-1855.
- [3] Bai LX, Song JN. Research advances in the mechanism of oxidative stress injury after traumatic brain injury. *Chin J Brain Dis Reh* 2013; **3**(5): 35-38.
- [4] Lu XY, Wang HD, Xu JG. Pretreatment with tert-butylhydroquinone attenuates cerebral oxidative stress in mice after traumatic brain injury. *J Surg Res* 2014; **188**(1): 206-212.
- [5] Rodríguez-Rodríguez A, Egea-Guerrero JJ, Murillo-Cabezas F. Oxidative stress in traumatic brain injury. *Curr Med Chem* 2014; **21**(10): 1201-1211.
- [6] Jayakumar AR, Rao KV, Panickar KS. Trauma-induced cell swelling in cultured astrocytes. *J Neuropathol Exp Neurol* 2008; **67**: 417-427.
- [7] Yu HL, Zhao TF, Wu H. Pinellia ternata lectin exerts a pro-inflammatory effect on macrophages by inducing the release of pro-inflammatory cytokines, the activation of the nuclear factor- $\kappa$ B signaling pathway and the overproduction of reactive oxygen species. *Int J Mol Med* 2015; **36**(4): 1127-1135.
- [8] Hong SH, Jeong HK, Han MH. Esculetin suppresses lipopolysaccharide-induced inflammatory mediators and cytokines by inhibiting nuclear factor- $\kappa$ B translocation in RAW 264.7 macrophages. *Mol Med Rep* 2014; **10**(6): 3241-3246.
- [9] Sinke AP, Jayakumar AR, Panickar KS. NF- $\kappa$ B in the mechanism of ammonia-induced astrocyte swelling in culture. *J Neurochem* 2008; **106**: 2302-2311.
- [10] Jayakumar R, Bethea JR, Tong XY. NF- $\kappa$ B in the mechanism of brain edema in acute liver failure: studies in transgenic mice. *Neurobiol Dis* 2011; **41**: 498-507.
- [11] Laird MD, Sukumari-Ramesh S, Swift AE. Curcumin attenuates cerebral edema following traumatic brain injury in mice: a possible role for aquaporin-4. *J Neurochem* 2010; **113**: 637-648.
- [12] Tóbon-Velasco JC, Cuevas E, Torres-Ramos MA. Receptor for AGEs (RAGE) as mediator of NF- $\kappa$ B pathway activation in neuroinflammation and oxidative stress. *CNS Neurol Disord Drug Targets* 2014; **13**(9): 1615-1626.
- [13] Siomek A. NF- $\kappa$ B signaling pathway and free radical impact. *Acta Biochim Pol* 2012; **59**(3): 323-331.
- [14] Yu XJ, Zhang DM, Jia LL. Inhibition of NF- $\kappa$ B activity in the hypothalamic paraventricular nucleus attenuates hypertension and cardiac hypertrophy by modulating cytokines and attenuating oxidative stress. *Toxicol Appl Pharmacol* 2015; **284**(3): 315-322.
- [15] Jiang P, Li C, Xiang Z. Tanshinone IIA reduces the risk of Alzheimer's disease by inhibiting iNOS, MMP-2 and NF- $\kappa$ Bp65 transcription and translation in the temporal lobes of rat models of Alzheimer's disease. *Mol Med Rep* 2014; **10**(2): 689-694.
- [16] Sticozzi C, Belmonte G, Meini A. L-1 $\beta$  induces GFAP expression in vitro and in vivo and protects neurons from traumatic injury-associated apoptosis in rat brain striatum via NF $\kappa$ B/Ca<sup>2+</sup>-calmodulin/ERK mitogen-activated protein kinase signaling pathway. *Neuroscience* 2013; **12**(252): 367-383.
- [17] Bubici C, Papa S, Dean K. Mutual crosstalk between reactive oxygen species and nuclear factor- $\kappa$ B: molecular basis and biological significance. *Oncogene* 2006; **25**(51): 6731-6748.
- [18] Luengo-Blanco M, Prando C, Bustamante J. Essential role of nuclear factor- $\kappa$ B for NADPH oxidase activity in normal and anhidrotic ectodermal dysplasia leukocytes. *Blood* 2008; **112**(4): 1453-1460.
- [19] Manea A, Manea SA, Gafencu AV. Regulation of NADPH oxidase subunit p22(phox) by NF- $\kappa$ B in human aortic smooth muscle cells. *Arch Physiol Biochem* 2007; **113**(4-5): 163-172.